**Insights into the microbial autotrophic potential of a shallow oligotrophic alpine pond**

# Insights into the microbial autotrophic potential of a shallow oligotrophic alpine pond

**Ilaria Mania[1], Martina Pellicciaro[1], Roberta Gorra[1]**

[1] Department of Agriculture, Forest and Food Sciences (DISAFA), University of Turin, Largo Braccini 2, 10095 Grugliasco (TO) - Italy

Corresponding author:

ilaria.mania@unito.it

Tel: +39 011 6708825

ORCID iD:

Mania I.: https://orcid.org/0000-0001-8723-0930

Pellicciaro M.: https://orcid.org/0000-0001-6778-723X

Gorra R.: https://orcid.org/0000-0002-7007-7113

**Abstract**

Carbon dioxide fixation is one of the most important biogeochemical processes worldwide, but our current understanding of the distribution of microbial autotrophy and its ecological significance in oligotrophic freshwater systems, and particularly in benthic habitats, is poor and mainly limited to photoautotrophic organisms. In this study, we investigated the autotrophic microbial communities inhabiting the sediments of a high elevation, oligotrophic freshwater pond in the North-Western Italian Alps. The abundance and distribution of three different forms of the RubisCO large-subunit gene were assessed in samples collected at different depths by qPCR, and correlations with sediment geochemical properties and total bacterial abundance were also examined. RubisCO forms *cbbL*G, *cbbL*R and *cbbM* were all detected, with abundances of 9.13-10.90, 6.93-8.77 and 6.75-7.93 Log copies per g of dry weight, respectively. For all of them interannual variability overcame depth-related variability. RubisCO genes abundance was strongly correlated with total bacterial abundance, and both of them were positively correlated with $Ca_2^+$ and $Mg_2^+$ concentration. These observations provide some first indications on the distribution of photo- and chemolithoautotrophic bacteria relying on the Calvin-Benson-Bassham (CBB) cycle for C fixation in alpine pond sediments, and suggest that they may represent an important component of the total benthic microbial community.

**Keywords**

Primary production

RubisCO

Sediments

qPCR

## Introduction

Benthic habitats have been reported to play a key role in supporting primary production in lentic ecosystems. In particular, their contribution tends to overcome those of pelagic habitats in shallow, oligotrophic water bodies: in these systems, where oligotrophic conditions penalize phytoplankton productivity, sediments act as a reservoir of nutrients, foraging benthic primary producers (Vadeboncoeur *et al*. 2003; Glud *et al*. 2009; Cremona *et al*. 2016; Zhang *et al*. 2020).

Submerged macrophytes and macroalgae are the most evident example of benthic primary producers, and the importance of their contribution in carbon, nitrogen and phosphorous immobilization has been described in different aquatic ecosystems (Dodds 2003; Vesterinen *et al.* 2016; Martinsen *et al.* 2017). However, other players may assume a major role in systems lacking aquatic vegetation: microalgae, diatoms, dinoflagellates and cyanobacteria, but also chemoautotrophic prokaryotes. Therefore, a better understanding of microbial primary production potential and dynamics is fundamental in order to develop a comprehensive view on the ecology of oligotrophic freshwater systems and to predict their potential response to perturbations. This is of great concern especially in fragile contexts such as alpine oligotrophic freshwater ecosystems, where the effects of climate change are expected to have strong impacts, for instance in terms of hydrological regime, water quality and trophic status (Beniston 2003; Slemmons *et al.* 2013; Redmond 2018).

A powerful tool that can be applied for the simultaneous detection and quantification of such organisms is the analysis of nucleic acids targeting functional genes linked to carbon fixation processes, such as ribulose-1,5 bisphosphate carboxylase/oxygenase (RubisCO) large subunit genes. RubisCO is one of the key enzymes in the Calvin–Benson cycle, the most widespread C fixation pathway in nature (Berg 2011). RubisCO exists in different forms, evolutionarily related but differing in structure, catalytical properties and substrate specificity (Tabita *et al.* 2008). The most common in Eukarya and Bacteria are form I and form II, whose large subunit is encoded by *cbbL* and *cbbM* genes respectively. Form I RubisCO can be further divided in two groups, based on the aminoacidic sequence of the enzyme large subunit (Watson and Tabita 1997): green-like, found in green algae, plants, *Cyanobacteria*, α-, β- and γ-*Proteobacteria*, and red-like, diffused in red algae and α- and β-*Proteobacteria*. Form II shows lower affinity to $CO_2$ and lower specificity than form I, suggesting a more ancient origin, and has been detected in Proteobacteria and dinoflagellates (Tabita 1999).

In this study we focused on an alpine clear water, oligotrophic pond characterized by the absence of evident macrophytes or macroalgal cover on sediments surface. The Col d'Olen Rock Glacier Pond is located in the NW Italian Alps, at the terminus of the homonymous rock glacier, covering an area of 1,600 $m^2$ and reaching a maximum depth of 3 m. The pond has been previously described in terms of hydrological dynamics (Colombo *et al.* 2017; Colombo *et al.* 2018), sediments geochemistry and prokaryotic diversity (Mania *et al.* 2019). In particular, Mania *et al.* (2019) proposed water depth as the main driver involved in microbial community shaping within the pond and reported several evidences suggesting the presence of a potential isle of primary production localized in the deepest area of the pond, such as higher levels of pH, DOC, TDN and $NH_4^+$ and presence of higher proportions of cyanobacterial sequences in deep versus shallow samples.

The aim of this study was therefore to study the portion of microbial-driven primary production linked to the CBB cycle in an alpine, periglacial context. Our objectives were to explore the autotrophic genetic potential of sediment microbial community through the quantification of RubisCO genes, and to test the influence of water depth and sediments geochemistry on the distribution of the different RubisCO forms.

## Materials and methods

A complete description of the sampling procedure is reported in Mania *et al.* 2019. Briefly, 10 cm sediment cores were aseptically collected from three sampling points in the Col d'Olen Rock Glacier Pond, located at different water depths (S1, S3 = 1 m, S2 = 3 m), for two consecutive years, during the snow-free season. At each sampling point three replicate samples were collected at a distance of approximately 50 cm. Total DNA was extracted and quantified as described in Mania *et al.* 2019. The abundance of genes encoding for RubisCO form I green-like and red-like (Paul *et al.* 2000; Selesi *et al.* 2005) and form II (Alfreider *et al.* 2003) was assessed by quantitative PCR (qPCR). qPCR reactions were performed using a Chromo4™ Real Time PCR Detection System (Bio-Rad Laboratories), in a reaction volume of 20 µl, including 10 µl of SsoAdvanced™ SYBR® Green Supermix (Bio-Rad), 0.3 µM of each primer and 2 µl of template DNA (diluted to less than 20 ng µl-1). Primer pairs and reaction conditions are summarised in Table 1. Each sample was analysed in triplicate, and product specificity was confirmed by melting curve analysis and visualisation on agarose gel. For standard curves setup PCR products were obtained from environmental samples or genomic DNA of reference organisms by applying the same cycling conditions used for qPCR with the addition of a final elongation step (Table 1). PCR products were purified with the PCR Extract Mini Kit (5 Prime), quantified by Qubit® (Life Technologies) and serially diluted in molecular grade water. The standard curves were analysed in triplicate, and reported R2 values higher than 0.99 and efficiencies of 66%, 52% and 68% for *cbbL* red-like, cbbL green-like and *cbbM* respectively. Gene abundance was compared among different sampling points and years by using 2-way ANOVA. Pearson's correlation coefficients were calculated to highlight significant relationships between RubisCO genes abundance and other parameters previously assessed on the same sediment samples (Mania *et al.* 2019): geochemical properties; bacterial 16S rRNA genes abundance, quantified by qPCR; cyanobacterial 16S rRNA genes proportion over total bacterial sequences, determined by 16S amplicon sequencing. All the statistical analyses were performed in R, version 3.4.0 (R Core Team 2017).

## Results and Discussion

The first objective of this study was to explore the potential of microbial communities in terms of C fixation in an alpine oligotrophic pond by assessing the abundance of the three most common forms of RubisCO genes. The abundance of RubisCO genes followed the order *cbbL*G > *cbbL*R > *cbbM*, ranging from 9.13 to 10.90, 6.93 to 8.77, and 6.75 to 7.93 Log copies per g of dry weight, respectively (Fig. 1).

Previous studies on markers of autotrophy have reported the prevalence of form I RubisCO among autotrophic communities in the water column of different oligotrophic aquatic ecosystems in cold areas

(Kong *et al.* 2012a; Kong *et al.* 2012b; Dolhi *et al.* 2015). Moreover, a recent survey on freshwater microbial communities in high-elevation catchments in the Tibetan Plateau (Kong *et al.* 2019) showed a prevalence of RubisCO sequences ascribable to the red-like form I over the green-like form I. In our system, the high levels of *cbbL*G genes could be connected with the presence of relevant proportions of cyanobacterial sequences described in the same samples by Mania *et al.* (2019), although a direct correlation between RubisCO genes abundance and cyanobacterial relative abundance was not found. Instead, given the low discrimination against $O_2$ and the poor affinity for $CO_2$ for form II RubisCO (Badger and Bek 2008), there is the possibility that a well-mixed and shallow pond is less favourable for the spread of microaerobic/anaerobic autotrophs.

An exact calculation of the proportion of $CO_2$-fixing bacteria on the total bacterial community is not achievable based on functional gene abundance data. Nevertheless, supposing that (i) the average copy number of 16S rRNA copies per genome in the bacterial cells is four to six and (ii) the average number of *cbb* operons in bacteria is two (Yuan *et al.* 2013; Lynn *et al.* 2017), then we can estimate that 2-3% of the bacteria in the pond sediments may have the potential to fix $CO_2$ through the CBB cycle.

Considering RubisCO genes distribution, no significant differences were reported among sediment samples collected in different areas of the pond, at different water depth (Fig. 1). This is possibly due to the limited variation in water depth, ranging from 1 to 3 m among the samples, and not apparently associated to variations in light and $O_2$ availability. However, significantly higher levels of all the genes were detected in 2015 (*cbbL*G: $F_{(1,18)} = 32.15$, $P < 0.001$; *cbbL*R: $F_{(1,18)} = 31.56$, $P < 0.001$; *cbbM*: $F_{(1,18)} = 23.17$, $P < 0.001$) if compared to 2016. Seasonal variations in benthic bacterial community structure and diversity have previously been shown to potentially overcome spatial variations, although information on microbial abundance is not available (Wan *et al.* 2017). In our case all the data refer to the late summer period, but it is interesting to highlight how in 2015 the early snowmelt led to a particularly prolonged snow-free season (Colombo *et al.* 2018), that might be related to the higher abundance of RubisCO and 16S rRNA bacterial genes. Indeed, analogous trends in total bacterial abundance have been described in the same samples by Mania *et al.* (2019), and the existence of a positive correlation between RubisCO and 16S rRNA genes copy number (Table 2) may indicate either that the variation in RubisCO genes abundance between 2015 and 2016 is ascribable to fluctuations in the overall bacterial population, or that autotrophic microorganisms actually represent a conspicuous component of the whole bacterial community.

Looking at the relationships existing between microbial markers and sediments geochemistry (Table 2), a significant positive correlation linked $Mg^{2+}$ and $Ca^{2+}$ to all the investigated RubisCO forms. This is not surprising, considering that $Mg^{2+}$ is a fundamental cofactor involved in RubisCO catalytic activity (Andersson 2008). Moreover, $Ca^{2+}$ availability has been shown to have an impact on cell viability, stress tolerance, maintenance of photosynthesis and RubisCO genes expression in Cyanobacteria (Tiwari et al. 2019). Other significantly positive correlations were found between $Mg^{2+}$, $Ca^{2+}$ and bacterial 16S rRNA gene abundance. As inorganic nutrients concentration may be a limiting factor for microbial communities in oligotrophic systems, also in this case we cannot clearly define which kind of relationship links total

bacterial abundance and the abundance of the autotrophic bacterial component.

Interestingly, the absence of depth-related trends in RubisCO genes abundance, particularly for the *cbbL*G form, seems to be in contrast with previous evidences suggesting the occurrence of higher proportions of *Cyanobacteria* in prokaryotic communities in the deepest area of the pond (Mania *et al.* 2019). The impossibility of assessing quantitative variations in taxa abundance within a community by using relative abundance data (Widder *et al.* 2016) could in part explain this discrepancy. However, the picture previously obtained from metabarcoding data was supported by the presence of a positive correlation between *Cyanobacteria* relative abundance and geochemical parameters potentially related to N fixation activity such as TDN and $NH_4^+$. Another potential explanation for the differences observed between relative abundance data and RubisCO gene trends can be found in the composition of the cyanobacterial community. Indeed, for the most abundant OTU in S2 samples an identification beyond the family level was not possible by using the SILVA database (Quast *et al.* 2013), and also when compared to accessions in the NCBI database it showed high sequence similarity with *Cyanobium* species but also with several uncultured *Cyanobacteria* detected in benthic ecosystems (Mania *et al.* 2019). Therefore, we can hypothesize that the primers used in this study, previously designed on a limited number of available RubisCO gene sequences *(*Paul *et al.* 2000*;* Alfreider *et al.* 2003; Selesi *et al.* 2005), may have failed to amplify this particular variant, potentially leading to a biased result in final *cbbL*G genes abundance. This is a common issue in molecular ecology studies relying on PCR-based techniques (Tremblay *et al.* 2015; Fischer *et al.* 2016), that could be overcome for instance by following a metagenomic approach, also suitable for the association of a predominant phylotype to correspondent functional genes.

## Conclusions

With this study we confirmed the genetic potential of the benthic microbial community of a shallow, oligotrophic alpine pond in terms of autotrophy based on the CBB cycle. All the investigated RubisCO forms, despite differing in overall abundance, showed a homogeneous distribution across the pond, not influenced by variations in water depth, while a significative interannual variability was reported. The strong link between RubisCO and bacterial 16S rRNA genes abundance, as well as the correlations with the same geochemical properties suggest that autotrophic organisms relying on the CBB cycle for C fixation may represent a relevant proportion of the total bacterial population in this kind of ecosystem.

## Conflicts of Interest

The authors declare no conflicts of interest

## Acknowledgements

This research did not receive any specific funding

## References


Alfreider, A., Vogt, C., Hoffmann, D., Babel, W. (2003). Diversity of ribulose-1,5-bisphosphate carboxylase/oxygenase large-subunit genes from groundwater and aquifer microorganisms. *Microbial Ecology* **45**, 317-328. doi: 10.1007/s00248-003-2004-9

Andersson, I. (2008). Catalysis and regulation in Rubisco. *Journal of Experimental Botany* **59**,1555-1568. doi: 10.1093/jxb/ern091

Badger, M. R., and Bek, E. J. (2008). Multiple Rubisco forms in proteobacteria: Their functional significance in relation to CO2 acquisition by the CBB cycle. *Journal of Experimental Botany* **59**,1525-1541. doi: 10.1093/jxb/erm297

Beniston, M. (2003). Climatic change in mountain regions: a review of possible impacts. *Climatic Change* **59**, 5-31. doi: 10.1023/A:1024458411589

Berg, I. A. (2011). Ecological aspects of the distribution of different autotrophic CO2 fixation pathways. *Applied and Environmental Microbiology* **77**, 1925-1936. doi: 10.1128/AEM.02473-10

Colombo, N., Gruber, S., Martin, M., Malandrino, M., Magnani, M., Godone, D., Freppaz, M., Fratianni, S., Salerno, F. (2018). Rainfall as primary driver of discharge and solute export from rock glaciers: The Col d'Olen Rock Glacier in the NW Italian Alps. *Science of the Total Environment* **639**, 316-330. doi: 10.1016/j.scitotenv.2018.05.098

Colombo, N., Sambuelli, L., Comina, C., Colombero, C., Giardino, M., Gruber, S., Viviano, G., Vittori Antisari, L., Salerno, F. (2017). Mechanisms linking active rock glaciers and impounded surface water formation in high-mountain areas. *Earth Surface Process and Landforms*. doi: 10.1002/esp.4257

Cremona, F., Laas, A., Arvola, L., Pierson, D., Nõges, P, Nõges, T. (2016). Numerical exploration of the planktonic to benthic primary production ratios in lakes of the Baltic Sea Catchment. *Ecosystems* **19**,1386-1400. doi: 10.1007/s10021-016-0006-y

Dodds, W. K. (2003). The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. *Journal of Phycology* **39**, 840-849. doi: 10.1046/j.1529-8817.2003.02081.x

Dolhi, J. M., Teufel, A. G., Kong, W., Morgan-Kiss, R. M. (2015). Diversity and spatial distribution of autotrophic communities within and between ice-covered Antarctic lakes (McMurdo Dry Valleys). *Limnology and Oceanography* **60**, 977-991. doi: 10.1002/lno.10071

Fischer, M.A., Güllert, S., Neulinger, S.C., Streit, W. R., Schmitz, R. A. (2016). Evaluation of 16S rRNA gene primer pairs for monitoring microbial community structures showed high reproducibility within and low comparability between datasets generated with multiple archaeal and bacterial primer pairs. *Frontiers in Microbiology* **7**, 1-15. doi: 10.3389/fmicb.2016.01297

Glud, R. N., Woelfel, J., Karsten, U., Kühl, M., Rysgaard, S. (2009). Benthic microalgal production in the Arctic: Applied methods and status of the current database. *Botanica Marina* **52**, 559-571. doi: 10.1515/BOT.2009.074

Kong, W., Dolhi, J. M., Chiuchiolo, A., Priscu, J., Morgan-Kiss, R. M. (2012a) Evidence of form II RubisCO (cbbM) in a perennially ice-covered Antarctic lake. *FEMS Microbiology Ecology* **82**, 491-500. doi: 10.1111/j.1574-6941.2012.01431.x

Kong, W., Liu, J., Ji, M., Yue, L., Kang, S., Morgan-Kiss, R. M. (2019). Autotrophic microbial community succession from glacier terminus to downstream waters on the Tibetan Plateau. *FEMS Microbiology Ecology* **95**, fiz074. doi: 10.1093/femsec/fiz074

Kong, W., Ream, D. C., Priscu, J. C., Morgan-Kiss, R. M. (2012b). Diversity and expression of RubisCO genes in a perennially ice-covered antarctic lake during the polar night transition. *Applied and Environmental Microbiology* **78**, 4358-4366. doi: 10.1128/AEM.00029-12

Lynn, T. M., Ge, T., Yuan, H., Wei, X., Wu, X., Xiao, K., Kumaresan, D., Yu, S. S., Wu, J., Whiteley, A. S. (2017). Soil carbon-fixation rates and associated bacterial diversity and abundance in three natural ecosystems. *Microbial Ecology* **73**, 645-657. doi: 10.1007/s00248-016-0890-x

Mania, I., Gorra, R., Colombo, N., Freppaz, M., Martin, M., Anesio, A. M. (2019). Prokaryotic diversity and distribution in different habitats of an alpine rock glacier-pond system. *Microbial Ecology* **78**, 70-84. doi: 10.1007/s00248-018-1272-3

Martinsen, K. T., Andersen, M. R., Kragh, T., Sand-Jensen, K. (2017). High rates and close diel coupling of primary production and ecosystem respiration in small, oligotrophic lakes. *Aquatic Sciences* **79**, 995-1007. doi: 10.1007/s00027-017-0550-3

Paul, J. H., Alfreider, A., Wawrik, B. (2000). Micro- and macrodiversity in rbcL sequences in ambient phytoplankton populations from the southeastern Gulf of Mexico. *Marine Ecology Progress Series* **198**, 9–18. doi: 10.3354/meps198009

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**, 590-596. doi: 10.1093/nar/gks1219

R Core Team (2017). 'R: A language and environment for statistical computing.' Available at https://www.R-project.org/.

Redmond, L. E. (2018). Alpine limnology of the Rocky Mountains of Canada and the USA in the context of environmental change. *Environmental Reviews* **26**, 231-238. doi: 10.1139/er-2017-0046

Selesi, D., Schmid, M., Hartmann, A. (2005). Diversity of green-like and red-like ribulose-1,5-bisphosphate carboxylase/oxygenase large-subunit lenes (cbbL) in differently managed agricultural soils. *Applied and Environmental Microbiology* **71**, 175-184. doi: 10.1128/AEM.71.1.175

Slemmons, K. E. H., Saros, J. E., Simon, K. (2013). The influence of glacial meltwater on alpine aquatic ecosystems: a review. *Environmental Science: Processes and Impacts* **15**, 1794-1806. doi: 10.1039/c3em00243h

Tabita, F. R. (1999). Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: A different perspective. *Photosynthesis Research* **60**, 1-28. doi: 10.1023/A:1006211417981

Tabita, F. R., Satagopan, S., Hanson, T.E., Kreel, N. E., Scott, S. S. (2008). Distinct form I, II, III, and IV Rubisco proteins from the three kingdoms of life provide clues about Rubisco evolution and structure/function relationships. *Journal of Experimental Botany* **59**:1515-1524. doi: 10.1093/jxb/erm361

Tiwari, A., Singh, P., Riyazat Khadim, S., Singh, A. K., Singh, U., Singh, P., Ashtana, R. K. (2019). Role of $Ca^{2+}$ as protectant under heat stress by regulation of photosynthesis and membrane saturation in Anabaena PCC 7120. *Protoplasma* **256**, 681-691. doi:10.1007/s00709-018-1328-8

Tremblay, J., Singh, K., Fern, A., Kirton, E. S., He, S., Woyke, T., Lee, J., Chen, F., Dangl, J. L., Tringe, S. G. (2015). Primer and platform effects on 16S rRNA tag sequencing. *Frontiers in Microbiology* **6**, 1-15. doi: 10.3389/fmicb.2015.00771

Vadeboncoeur, Y., Jeppesen, E., Vander Zanden, M. J., Schierup, H-H., Christoffersen, K., Lodge, D. M. (2003). From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. *Limnology and Oceanography* **48**:1408-1418. doi: 10.4319/lo.2003.48.4.1408

Vesterinen, J., Devlin, S. P., Syväranta, J., Jones, R. I. (2016). Accounting for littoral primary production by periphyton shifts a highly humic boreal lake towards net autotrophy. *Freshwater Biology* **61**:265-276. doi: 10.1111/fwb.12700

Wan, Y., Bai, Y., He, J., Zhang, Y., Li, R., Ruan, X. (2017). Temporal and spatial variations of aquatic environmental characteristics and sediment bacterial community in five regions of Lake Taihu. *Aquatic Ecology* **51**, 343-358. doi: 10.1007/s10452-017-9621-8

Watson, G. M. F., and Tabita, F. R. (1997). Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: a molecule for phylogenetic and enzymological investigation. *FEMS Microbiology Letters* **146**, 13-22. doi: 10.1111/j.1574-6968.1997.tb10165.x

Widder, S., Allen, R. J., Pfeiffer, T., Curtis, T. P., Wiuf, C., Sloan, W. T., Cordero, O. X., Brown, S. P., Momeni, B., Shou, W., Kettle, H., Flint, H. J., Haas, A. F., Laroche, B., Kreft, J-U., Rainey, P. B., Freilich, S., Schuster, S., Milferstedt, K., van der Meer, J. R., Groβkopf, T., Huisman, J., Free, F., Picioreanu, C., Quince, C., Klapper, I., Labarthe, S., Smets, B. F., Wang, H., Isaac Newton Institute Fellows, Soyer, O. K. (2016). Challenges in microbial ecology: Building predictive understanding of community function and dynamics. The *ISME Journal* **10**, 2557-2568. doi: 10.1038/ismej.2016.45

Yuan, H., Ge, T., Zou, S., Wu, X., Liu, S., Zhou, P., Chen, X., Brookes, P., Wu, J. (2013). Effect of land use on the abundance and diversity of autotrophic bacteria as measured by ribulose-1,5-biphosphate

carboxylase/oxygenase (RubisCO) large subunit gene abundance in soils. *Biology and Fertility of Soils* **49**, 609-616. doi: 10.1007/s00374-012-0750-x

Zhang, H., Yan, M., Huang, T., Huang, X., Yang, S., Li, N., Wang, N. (2020). Water-lifting aerator reduces algal growth in stratified drinking water reservoir: Novel insights into algal metabolic profiling and engineering applications. *Environmental Pollution* **266**. doi: 10.1016/j.envpol.2020.115384

297 **Table 1 Primer pairs, amplification conditions and standard organisms used in this study**

| Primer pair | Amplification protocols | Reference | DNA for standard preparation |
|---|---|---|---|
| *cbbM* F<br>*cbbM* R | 95 °C 4 min;<br>35 cycles: 95 °C 45 s,<br>57 °C 45 s, 72 °C 1 min,<br>85 °C 10 s;<br>(72 °C 10 min)[a] | Alfreider *et al.*, 2003 | *Thiomonas intermedia* DSM 18155 |
| *cbbL*R F<br>*cbbL*R R | 95 °C 4 min;<br>32 cycles: 95 °C 1 min,<br>57 °C 1 min, 72 °C 1 min 30 s,<br>85 °C 10 s;<br>(72 °C 10 min)[a] | Selesi *et al.*, 2005 | Environmental isolate cultured from sample S2.3.15 |
| *cbbL*G F<br>*cbbL*G R | 95 °C 3 min;<br>35 cycles: 95 °C 1 min,<br>52 °C 1 min, 72 °C 1 min 30 s,<br>85 °C 10 s;<br>(72 °C 10 min)[a] | Paul *et al.*, 2000 | Environmental sample S2.3.15 |

[a] Final elongation was excluded from qPCR protocol and used only in PCR reaction for standard preparation.

298

299

300 **Table 2 Correlation analysis (n = 18) among RubisCO genes abundance assessed in this study and**
301 **bacterial 16S rRNA genes abundance (16S rRNA Bact), *Cyanobacteria* relative abundance (Cyano) and**
302 **geochemical properties (data from Mania et al. 2019).**

303 Pearson's correlation coefficients in bold indicate statistical significance Significance level: *P<0.05,
304 **P>0.01, ***P<0.001

| | *cbbL*G | *cbbL*R | *cbbM* | 16S rRNA Bact | Cyano |
|---|---|---|---|---|---|
| C/N | -0.222 | -0.080 | -0.261 | -0.155 | -0.199 |
| DOC | 0.338 | 0.250 | -0.029 | 0.436 | 0.381 |
| TDN | 0.012 | 0.041 | -0.238 | 0.133 | **0.560*** |
| $NH_4^+$ | -0.269 | -0.319 | -0.434 | -0.169 | **0.669**** |
| $NO_3^-$ | 0.213 | 0.078 | 0.300 | -0.035 | -0.141 |
| pH | 0.004 | -0.132 | -0.356 | 0.053 | 0.413 |
| $Mg^{2+}$ | **0.576*** | **0.663**** | **0.504*** | **0.566*** | -0.166 |
| $Ca^{2+}$ | **0.576*** | **0.651**** | 0.447 | **0.573*** | -0.164 |
| $K^+$ | **0.246*** | **0.381*** | 0.337 | 0.343 | -0.141 |
| $Na^+$ | 0.417 | 0.482 | **0.573*** | 0.463 | -0.027 |
| Si | 0.133 | 0.001 | -0.086 | 0.162 | 0.321 |
| $Cl^-$ | 0.232 | 0.382 | **0.553*** | 0.237 | -0.280 |
| $PO_4^{3-}$ | -0.269 | -0.266 | -0.323 | -0.416 | -0.213 |
| $SO_4^{2-}$ | 0.378 | **0.528*** | **0.483*** | 0.394 | -0.274 |
| *cbbL*G | | **0.944***** | **0.740***** | **0.898***** | -0.242 |
| *cbbL*R | | | **0.795***** | **0.913***** | -0.080 |
| *cbbM* | | | | **0.680**** | -0.400 |
| 16S rRNA Bact | | | | | -0.256 |

305

306 **Figure captions**

307 **Fig. 1** Abundance of different RubisCO large subunit genes in sediment samples collected across the Col
308 d'Olen Rock Glacier Pond. Different colours correspond to different sampling years (dark grey: 2015; light
309 grey: 2016). Each bar represents the average of three field replicates, and error bars display the standard
310 error. Different letters indicate significant differences ($P < 0.05$) assessed by 2-way ANOVA

311

312